REMARKS

The Office Action of May 17, 2006, has been received and reviewed.

Claims 1-27 are currently pending and under consideration in the above-referenced application, each standing rejected.

Reconsideration of the above-referenced application is respectfully requested.

FRET Quenching vs. Non-FRET Quenching

Non-FRET-Quenching

Lakowicz, Principles of Fluorescence Spectroscopy (2nd Edition) (hereinafter "Lakowicz"), a copy of which has already been provided to the Office, notes that non-fluorescence resonance energy transfer ("non-FRET") quenching can occur through a variety of mechanisms, none of which are considered to be non-radiative or radiationless energy transfer, or any type of resonance energy transfer. Non-FRET quenching of a fluorophore by another fluorophore or by a non-fluorophore quencher does not rely on resonance energy transfer. Instead of resonance energy transfer, non-FRET static or ground-state quenching involves formation of a ***non-fluorescent complex*** between a fluorophore and quencher that occurs ***while the fluorophore is in the ground state***.

When non-FRET static or ground-state quenching occurs, light is absorbed by the fluorophore-quencher complex, but ***there is no fluorescence emission***. Because of the ground-state interaction, there is however a change in the absorbance spectrum of the complex compared to unbound or free fluorophore. When the non-fluorescent complex is disrupted, non-FRET quenching no longer occurs, and the fluorophore is able to fluoresce. Thus, non-FRET quenching is an all-or-none phenomenon determined by whether the complex is associated (non-fluorescent) or dissociated (fluorescent). In contrast, resonance energy transfer (FRET) is a graded response that depends on the distance between donor and acceptor fluorophores.

Collisional or dynamic quenching is another form of non-FRET quenching that can occur during the excited state of the fluorophore. Quenching occurs when the excited fluorophore is deactivated by collision or diffusive encounter with a quencher molecule. This form of quenching requires that the fluorophore be in the excited state. The contact with the quencher

returns the fluorophore to the ground state ***without emission of a photon***. The mechanism of dynamic quenching depends on the fluorophore-quencher pair, and can include electron transfer, spin-orbit coupling and intersystem crossing. Collisional quenching thus requires the absorption of a photon of light by the fluorophore, the consequent formation of the fluorophore excited state, and collision with a small molecule quencher during the excited state. The degree of collisional quenching is determined in large part by the concentration of quencher molecule, which determines the frequency of collisions with the excited-state fluorophore. In contrast to ground-state (static) quenching, there is typically no change in the absorption spectrum of the fluorophore with collisional quenching.

FRET-Quenching

Lakowicz teaches that non-radiative or radiationless *energy transfer* (as occurs in fluorescence resonance energy transfer ("FRET")), occurs between a donor and acceptor pair that are within a certain critical distance. In addition, the absorption (excitation) spectrum of the acceptor fluorophore must overlap with the emission spectrum of the donor fluorophore. The greater the spectral overlap, the more efficient the energy transfer between donor and acceptor. There is no emission of a photon during this process, thus the process is termed radiationless or non-radiative energy transfer. Energy transfer to a donor results in quenching of donor fluorescence, but an increase in fluorescence emission of the acceptor fluorophore.

In addition to spectral overlap, the degree of FRET-quenching between two fluorophores depends upon the distance between the two fluorophores. Specifically, the degree of FRET-quenching is the distance between the donor and acceptor fluorophores, raised to the inverse sixth power.

Claim Rejections under 35 U.S.C. § 112

Claims 1-27 have been rejected under 35 U.S.C. § 112, first paragraph, because the specification of the above-referenced application allegedly does not provide an adequate written description for doubly labeled compounds that have at least a part of their fluorescence quenched through ground state interaction.

It has been asserted that the specification of the above-referenced application is not enabling because it does not present fluorescence lifetime data. It is respectfully submitted that when quenching is due to physical interactions between dye molecules, the quenched dye does not fluoresce. Therefore, a non-FRET quenched dye cannot have a fluorescence lifetime. To include such data would comprise mischaracterization of the phenomena that occur as ground state interactions take place between fluorescent dye dimers.

Moreover, it is respectfully submitted that the specific examples that have been provided in the specification of the above-referenced application would enable one of ordinary skill in the art to practice the inventions recited in each of the claims of the above-referenced application. In particular, it would be evident to one of ordinary skill in the art from the disclosure of the above-referenced application that a system in which two molecules that physically associate with one another to quench fluorescence or physically dissociate from one another to dequench fluorescence would work regardless of the length of the molecule, provided that the molecules could be attached at locations along the molecule that would allow for such physical interaction. Moreover, it is respectfully submitted that one of ordinary skill in the art would have a ready understanding of the tertiary and quaternary structures of molecules with which they might want to use dye dimers in the manner that has been disclosed and claimed, as well as the locations on such molecules where such dye dimers could be attached prior to covalent modification of the molecules. As such, it is respectfully submitted that the disclosure of the above-referenced application provides one of ordinary skill in the art with a sufficient understanding of the inventions recited in the claims to enable one of ordinary skill in the art to practice the invention in a wide variety of molecular systems.

In view of the foregoing, as well as in view of the remarks that were previously presented in the above-referenced application with respect to the 35 U.S.C. § 112, first paragraph, rejections of claims 1-27, it is respectfully submitted that, from the disclosure of the above-referenced application, one of ordinary skill in the art would have a sufficient understanding of the claimed subject matter to enable one of ordinary skill in the art to practice the claimed invention with molecules that have not been discussed in the specification of the

above-referenced application. Accordingly, withdrawal of the 35 U.S.C. § 112, first paragraph, rejections of claims 1-27 is respectfully solicited.

Rejections under 35 U.S.C. § 102

Claims 1, 2, 5-7, 9, 11, 15, and 21 stand rejected under 35 U.S.C. § 102(b).

A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single reference which qualifies as prior art under 35 U.S.C. § 102. *Verdegaal Brothers v. Union Oil Co. of California*, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987). The identical invention must be shown in as complete detail as is contained in the claim. *Richardson v. Suzuki Motor Co.*, 9 USPQ2d 1913, 1920 (Fed. Cir. 1989).

With respect to inherency, M.P.E.P. § 2112 provides:

> The fact that a certain result or characteristic may occur or be present in the prior art is not sufficient to establish inherency of that result or characteristic. *In re Rijckaert,* 9 F.3d 1531, 1534, 28 USPQ2d 1955, 1957 (Fed. Cir. 1993) . . . 'To establish inherency, the extrinsic evidence 'must make clear that the missing descriptive matter is necessarily present in the thing described in the reference, and that it would be so recognized by persons of ordinary skill . . .'' *In re Robertson,* 169 F.3d 743, 745, 49 USPQ2d 1949, 1950-51 (Fed. Cir. 1991).

Odom

Claims 1, 2, 5-7, 9, 11, 15, and 21 stand rejected under 35 U.S.C. § 102(b) for reciting subject matter which is purportedly anticipated by that described in Odom, O.W., et al., "An apparent conformational change in $tRNA^{Phe}$ that is associated with the peptidyl transferase reaction," Biochemie 69:925-38 (1987) (hereinafter "Odom").

Independent claim 1, as amended and presented herein, is drawn to a biomolecular substrate that includes a core molecular backbone and first and second dyes that are covalently attached to the core molecular backbone. When the biomolecular substrate is not covalently modified, the first and second dyes ***physically associate*** to form a quenched intramolecular dye dimer. Such quenching is effected at least in part through a non-fluorescence resonance energy

transfer (non-FRET) mechanism. When the biomolecular substrate is covalently modified, the first and second dyes ***physically dissociate***, which results in quenching of at least one of the dyes.

Independent claim 15, as amended and presented herein, recites a method for assaying covalently biomolecular modification. The method of independent claim 15 includes providing a sample that includes a core molecular backbone, as well as first and second dyes covalently attached to the core molecular backbone. Non-FRET induced changes in fluorescent or absorbance characteristics of the biomolecular substrate, which occur with the first and second dyes ***physically associate*** with one another and ***physically dissociate*** from one another, may be quantified to determine whether or to the biomolecular substrate has been covalently modified.

Non-FRET mechanisms for inducing changes in fluorescence or absorbance characteristics of a fluorescent dye include so-called "static quenching," or "ground state quenching," interactions between the fluorescent dye and another molecule (*e.g.,* another dye), which is known in the art as a "quencher." Ground state quenching includes ***physical interaction***, such as binding, between a fluorescent dye molecule, or fluorophore, and a quencher to form a nonfluorescent complex between the fluorophore and the quencher. Binding of the quencher to the fluorophore frequently perturbs the absorption spectrum of the fluorophore. Thus, ***the fluorophore cannot fluoresce.*** Moreover, upon absorbing light, this non-FRET, nonfluorescent complex immediately returns to the ground state without emitting a photon. *See, e.g.,* The Glen Report, Vol. 17, No. 1, September 2004, a copy of which is enclosed for the sake of convenience (hereinafter "the Glen Report").

FRET is known to those of ordinary skill in the art to be fundamentally different from non-FRET mechanisms, such as ground state quenching. Specifically, FRET is a photophysical process in which ***a first fluorophore***, which is commonly referred to as a "donor molecule," is excited and, thus, ***fluoresces***. ***The fluorescence***, or energy, emitted by the donor molecule ***is*** then ***absorbed*** by an "acceptor molecule." The amount of quenching that occurs as the acceptor molecule absorbs energy from the donor molecule depends on a variety of factors, including the overlap of the emission spectrum of the donor molecule and the absorption spectrum of the acceptor molecule, the quantum yield of the donor molecule, the relative orientations of the donor and acceptor molecules, and the physical distance between the donor and acceptor

molecules. *See, e.g.,* the Glen Report and http://etd/caltech.edu/etd/available/etd-05112004-101833/unrestricted/(06)ch4_Title_text.pdf, titled "Chapter 4: Fluorescence Resonance Energy Transfer (FRET) by Minor Groove-Associated Cyanine-Polyamide Conjugates," published May 11, 2004, a copy of which was previously provided.

As there may be no emission by a fluorophore when non-FRET quenching occurs, non-FRET quenching does not necessarily involve FRET.

The description of Odom relates to use of a pair of fluorescent molecules to detect conformational changes in $tRNA^{Phe}$. In particular, the methods that are described in Odom include evaluation of $tRNA^{Phe}$ molecules that were labeled with both DCCH (coumarin) and FITC (fluorescein), where DCCH is the donor and FITC is the acceptor in a system in which "energy transfer by donor quenching" occurred. Odom, page 931. Thus, the description of Odom is clearly limited to a biomolecular substrate that is labeled with dyes that are quenched through FRET, as well as to an assay method that includes use of a FRET-quenched dye pair.

Further, as the disclosure of Odom is limited to FRET quenching, and ground state quenching does not necessarily involve FRET, Odom does not inherently describe a biomolecular substrate that is configured for non-FRET quenching or a method in which non-FRET quenching may occur.

Therefore, Odom does not anticipate either the biomolecular substrate of independent claim 1 or the assay method of independent claim 15. Accordingly, under 35 U.S.C. 102(b), the subject matter recited in both independent claim 1 and independent claim 15 is allowable over the subject matter described in Odom.

Each of claims 2, 5-7, 9, and 11 is allowable, among other reasons, for depending directly from claim 1, which is allowable.

Claim 21 is allowable, among other reasons, for depending directly from claim 15, which is allowable.

Withdrawal of the 35 U.S.C. § 102(b) rejections of claims 1, 2, 5-7, 9, 11, 15, and 21 is respectfully solicited.

Gildea

Claims 1-2, 5-7, 9, 11, 15, and 21 are rejected under 35 U.S.C. § 102(e) as allegedly being anticipated by the disclosure of U.S. Patent 6,485,901 to Gildea et al. (hereinafter "Gildea").

Gildea discloses a non-naturally occurring polyamide probe called a peptide nucleic acid (PNA) which can hybridize to DNA and RNA. Gildea, col. 5, lines 29-34. The PNA probe includes donor and acceptor moieties located at opposite ends of the probe. The PNA probe targets a complementary DNA or RNA sequence and, upon hybridization to the target sequence by hydrogen bonding, a detectable signal from at least one acceptor or donor moiety can be used to monitor or quantitate the hybridization event.

Notably, several passages in Gildea may cause confusion with regard to the photophysical mechanisms that are disclosed in Gildea and those recited in the claims of the above-referenced application. These passages are located at col. 1, lines 17-41, and col. 13, lines 14-40, of Gildea.

In particular, col. 1, lines 17-41, of Gildea provides that FRET is "also known as non-radiative energy transfer" and that non-FRET interactions are "also known as radiationless energy transfer." The statement that FRET is known as "non-radiative energy transfer" is true. However, this term is used synonymously by those of skill in the art with the term "radiationless energy transfer." The statement in Gildea that non-FRET quenching is "radiationless" is not true.

In this regard, Lakowicz notes that non-FRET quenching can occur through a variety of mechanisms, none of which is considered to be "radiationless energy transfer." Non-FRET quenching, or static or ground-state quenching, involves formation of a non-fluorescent complex between a fluorophore and quencher that occurs while the fluorophore is in the ground state. It does not rely on diffusion or molecular collisions, and *there is no energy transfer.*

Lakowicz also teaches that non-radiative or radiationless *energy transfer* (as occurs in collisional or dynamic quenching) occurs when an excited fluorophore is deactivated by collision or diffusive encounter with a quencher molecule. This form of quenching requires that the fluorophore be in the excited state. The contact with the quencher returns the fluorophore to the ground state without emission of a photon. The mechanism of dynamic quenching depends on

the fluorophore-quencher pair, and can include electron transfer, spin-orbit coupling and intersystem crossing.

The disclosure of Gildea is limited to "radiationless energy transfer." There is no energy transfer when ground state interactions occur between a fluorescent dye molecule and its corresponding quencher, as recited in amended independent claims 1 and 15. It is, therefore, respectfully submitted that Gildea does not expressly or inherently describe a biomolecular substrate that includes a core molecular backbone and first and second dyes that are covalently attached to the core molecular backbone and that may ***physically associate*** with one another to form a ground state-quenched dimer and ***physically dissociate*** from each other to dequench.

In any event, Gildea includes no express or inherent description of covalent modification of a biomolecular substrate. Rather, the description of Gildea is limited to nucleotide hydrogen bonding between a PNA probe and a target DNA or RNA.

Furthermore, from the inaccuracies that have been identified with respect to the disclosure of Gildea (*i.e.,* that non-FRET interactions involve "radiationless energy transfer"), one of ordinary skill in the art couldn't be reasonably certain, without undue experimentation, that non-FRET interactions actually occur in the molecules that are described in Gildea.

Therefore, Gildea does not anticipate either the biomolecular substrate of amended independent claim 1 or the assay method of amended independent claim 15. Accordingly, under 35 U.S.C. § 102(e), the subject matter recited in both amended independent claim 1 and amended independent claim 15 is allowable over the subject matter described in Gildea.

Each of claims 2, 5-7, 9, and 11 is allowable, among other reasons, for depending directly from claim 1, which is allowable.

Claim 21 is allowable, among other reasons, for depending directly from the allowable claim 15.

It is respectfully requested that the 35 U.S.C. § 102(b) rejections of claims 2, 5-7, 9, 11, 15, and 21 be withdrawn, and that each of these claims be allowed.

Rejections under 35 U.S.C. § 103(a)

Claims 1-27 have been rejected under 35 U.S.C. § 103(a).

The standard for establishing and maintaining a rejection under 35 U.S.C. § 103(a) is set forth in M.P.E.P. § 706.02(j), which provides:

> To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991).

Blumenthal, Odom, and Tyagi

Claims 1-27 stand rejected under 35 U.S.C. § 103(a) for reciting subject matter which is assertedly unpatentable over that taught in Blumenthal, D.K., "Development and Characterization of Fluorescently-Labeled Myosin Light Chain Kinase Calmodulin-Binding Domain Peptides," Molecular and Cellular Biochemistry, 1271128: 45-50 (1993) (hereinafter "Blumenthal"), Odom, and either WO 97139008 of Tyagi or U.S. Patent 6,150,097 to Tyagi (hereinafter "Tyagi").

Blumenthal teaches biomolecular substrates that include a single dye (acrylodan) for measuring changes in the conformation of a calmodulin binding domain of a peptide when the peptide binds calmodulin. In one of the methods, the analyzed peptide included naturally occurring tryptophan residues and was employed as a FRET donor molecule, while the acrylodan acted as the FRET acceptor molecule. When calmodulin binds *noncovalently* to the peptide, a conformational change in the peptide occurred, which decreased the distance between the tryptophan and the acrylodan and, thus, caused an increase in FRET quenching. Blumenthal does not include any teaching or suggestion of use of non-FRET quenching to determine whether or not a conformational change in the peptide occurred, or that non-FRET quenching may be used

to determine whether a covalent, non-cleavage modification of a biomolecular substrate has occurred.

The teachings of Odom have been summarized above. Most notably, the teachings of Odom are limited to use of a dye pair in which changes in FRET quenching are indicative of conformational changes in $tRNA^{Phe}$ that may occur as a ribosome-bound AcPhe-tRNA is deacylated to produce a $tRNA^{Phe}$ by the peptidyl transferase reaction. Odom, p. 937, first column. During the peptidyl transferase reaction, deacylation of the ribosome-bound AcPhe-tRNA involves the cleavage of covalent bonds. First, the peptidyl-tRNA is cleaved from the carboxyl end of a growing peptide chain and then peptide bond formation proceeds with the aminoacyl-tRNA. The covalent bond cleavage occurs as a result of nucleophilic attack by the lone pair of electrons on the amino nitrogen of the aminoacyl-tRNA on the carbonyl carbon that attaches the growing polypeptide chain to the peptidyl-tRNA molecule in the P site of the ribosome. Like Blumenthal, Odom does not include any teaching or suggestion of use of non-FRET quenching to determine whether or not a biomolecular substrate has been modified or, more specifically, that non-FRET quenching may be used to determine when a biomolecular substrate has been covalently modified but not cleaved.

Tyagi teaches the use of "Molecular Beacon" oligonucleotide probes to monitor binding of oligonucleotide probes labeled with two fluorescent dyes or a fluorophore and a quencher to target sequences in nucleic acids. While fluorescence of the fluorophore may be quenched when the quencher "touches" the fluorophore and, thus, ground state quenching may occur, such quenching only occurs because a hairpin, which is ***unique to nucleic acids***, has been designed into the nucleic acid. When the nucleic acid binds (by hydrogen bonding, not covalent modification) to a complementary nucleic acid sequence, the hairpin unwinds, causing dissociation of the dyes and, thus, allowing for fluorescence of the fluorophore. As such, Tyagi lacks any teaching or suggestion of use of non-FRET quenching to determine whether or not a biomolecular substrate has been covalently modified without being cleaved.

It is respectfully submitted that there are a number or reasons that the teachings of Blumenthal, Odom, and Tyagi do not support a *prima facie* case of obviousness against any of claims 1-27.

First, it is respectfully submitted that Blumenthal, Odom, and Tyagi, taken in any combination or individually, do not teach or suggest each and every element of any of claims 1-27.

None of Blumenthal, Odom, or Tyagi, taken either together or separately, teaches or suggests a biomolecular substrate that is covalently modified without being cleaved, as recited by independent claim 1; a method in which a biomolecular substrate is covalently modified without being cleaved, as recited in independent claims 15, 25, and 27; or a kit including a biomolecular substrate and a dye that, when the biomolecular substrate is covalently modified without being cleaved, dissociates from another dye to reduce quenching by ground-state interactions between the dyes, as recited in independent claim 26.

In regard to claims 23 and 24, it is respectfully affirmed that independent claim 23 is allowable since none of Blumenthal, Odom, or Tyagi teaches or suggests a method for assaying protein kinase activity, let alone various aspects of such a method, including provision of a biomolecular substrate that includes a KID peptide sequence or a pair of molecules that, when the biomolecular substrate is not covalently modified, form an intermolecular dye dimer, but, when the biomolecular substrate is phosphorylated, dissociate to reduce quenching between the pair of molecules.

Claim 24 is allowable, among other reasons, for depending directly from claim 23, which is allowable.

Second, without the benefit of hindsight provided by the claims of the above-referenced application, it is respectfully submitted that one of ordinary skill in the art wouldn't have been motivated to combine teachings from Blumenthal, Odom, and Tyagi in the manner that has been asserted. This is because one of ordinary skill in the art would have no reason to expect that the nucleic acid hairpin structure that is instrumental in properly orienting the fluorophore and quencher of Tyagi could be made in any other type of biomolecular substrate, or that such a structure, even if obtainable, would unfold or unwind upon covalent modification of the

biomolecular substrate in such a way as to result in dissociation of the fluorophore and its quencher.

For these reasons, the teachings of Blumenthal, Odom, and Tyagi do not support a *prima facie* case of obviousness against any of claims 1-27. Accordingly, it is respectfully submitted that, under 35 U.S.C. § 103(a), the subject matter recited in each of claims 1-27 is allowable over the teachings of Blumenthal, Odom, and Tyagi.

Macala, Shultz, or Ventura in View of Blumenthal and Odom or Tyagi

Claims 1-27 stand rejected under 35 U.S.C. § 103(a) for reciting subject matter that is allegedly unpatentable over the subject matter taught in Macala, L.J., et al., "Measurement of cAMP-Dependent Protein Kinase Activity Using a Fluorescent-Labeled Kemptide," Kidney International, 54: 1746-50 (1998) (hereinafter "Macala"), U.S. Patent 5,580,747 to Schultz et al. (hereinafter "Schultz"), or Ventura, C., et al., "Phorbol Ester Regulation of Opioid Peptide Gene Expression in Myocardial Cells," The Journal of Biological Chemistry, 270(50): 301 15-20 (1995) (hereinafter "Ventura"), in view of teachings from Blumenthal and Odom or Tyagi.

Although several "difficulties" have been identified by the Office (see page 10 of the final Office Action), it is respectfully submitted that "placement of two dyes along the backbone at positions that they will interact and quench in one configuration of the backbone and not interact or quench in the second configuration of the backbone" is really the only difficulty in practice. For this reason, the use of a combinatorial library of compounds is disclosed, allowing one of ordinary skill in the art to select from dye-quencher pairs that can be used at different locations on a particular molecule to facilitate distinction between unmodified and covalently modified versions of the molecule.

Macala teaches a protein kinase assay that uses a protein kinase substrate labeled with *one fluorophore*. The Macala assay includes electrophoretically separating the phosphorylated product from the nonphosphorylated fluorescent substrate using agarose gels. The Macala assay is not a homogenous assay that can be use for continuous, high throughput screening of enzyme activity, nor for use in measuring enzyme activity in living cells. As acknowledged by the Office, Macala does not teach or suggest a substrate having two dyes attached or a library of compounds.

Schultz teaches a fluorescent assay using a modified substrate labeled with a single dye. The Schultz assay requires the separation of the reaction products from the substrate by electrophoresis, chromatography, or extraction. Like Macala, this assay is not a homogenous assay that can be use for continuous, high throughput screening of enzyme activity, nor for use in measuring enzyme activity in living cells. As acknowledged by the Office, Schultz does not teach or suggest a substrate having two dyes attached or a library of compounds.

Ventura, likewise, teaches a single-labeled fluorescent protein kinase peptide substrate. The single-labeled peptide shows a *20% decrease* in activity when it is fully phosphorylated. Because the fluorescence change is measured in a decrease with a maximum change of 20%, it is difficult to measure low levels of phosphorylation. As acknowledged by the Office, Ventura does not teach or suggest a substrate having two dyes attached or a library of compounds.

Blumenthal teaches the use of acrylodan-labeled synthetic peptides to study calmodulin-peptide interactions. The peptides are based on the calmodulin-binding domain of myosin light chain kinase. The paper describes fluorescence studies using these peptides, including studies where binding of acrylodan labeled peptides to calmodulin were measured by changes in fluorescence intensity due to changes in solvent exposure of the acrylodan, changes in fluorescence anisotropy due to changes in rotational diffusion, and changes in fluorescence intensity due to FRET between the intrinsic tryptophan donor and the acrylodan acceptor. The *noncovalent* binding of the peptide to calmodulin induces helix formation in the peptide that brings the donor-acceptor pair within FRET distance, causing an increase in fluorescence emission of the acceptor. None of the fluorescence studies in Blumenthal involved covalent modification or measurement of protein kinase activity, nor did they involve ground state quenching of fluorescence.

The teachings of Odom and Tyagi have been summarized above. Most notably, and as discussed previously, neither Odom nor Tyagi teaches or suggests a biomolecular substrate that is covalently modified *without being cleaved.*

With respect to the purported combination of teachings from Macala, Shultz, Ventura, Blumenthal, and Odom, it is respectfully submitted that none of these references teaches or suggests a biomolecular substrate that includes a core molecular backbone and a pair of dyes that

undergo ground-state interactions prior to covalent modification of the backbone and dissociate from one another following covalent modification of the backbone without being cleaved. Moreover, none of these references teaches or suggests use of such a biomolecular substrate in an assay. Thus, none of these references teaches or suggests each and every element of any of claims 1-27.

In addition, since Macala, Shultz, Ventura, Blumenthal, and Odom, taken together, do not teach or suggest each and every element of any of claims 1-27, one of ordinary skill in the art would have no reason to expect the purported combination of teachings from these references to be successful.

Moreover, it is respectfully submitted that, without the benefit of hindsight that the claims of the above-referenced application provide to the Office, one of ordinary skill in the art wouldn't have been motivated to combine teachings from Macala, Shultz, or Ventura, with teachings from Blumenthal and Odom. This is because peptides are relatively unstructured in solution, as opposed to nucleic acids, such as those taught in Odom, which self-hybridize in solution to form hair-pin structures between complementary internal sequences. As such, one of ordinary skill in the art would have had no reason to expect that attaching a dye near each end of a peptide might generate a reagent that would be useful for monitoring non-cleavage, covalent modifications to a peptide by non-FRET quenching.

Furthermore, it is respectfully submitted that one of ordinary skill in the art would have had no reason to combine the teachings of these references. Of these references, only Tyagi teaches fluorescence quenching between a pair of dye molecules. The teachings of Tyagi are limited to reductions in fluorescence quenching as a single stranded probe nucleic acid hybridizes with another single stranded nucleic acid, *which is a noncovalent modification* of the probe nucleic acid. While Macala, Shultz, Ventura, and Blumenthal merely teach use of single or non-interacting fluorescent molecules on a core molecular substrate. None of Macala, Shultz, Ventura, or Blumenthal teaches or suggests that a pair of dyes may be used to indicate covalent changes to a core molecular substrate. Further, one of ordinary skill in the art wouldn't have been motivated to combine teachings from Tyagi with those of Macala, Shultz, Ventura, or Blumenthal due to stark dissimilarities between the peptide substrates of Macala, Shultz,

Ventura, and Blumental and the nucleotide substrates of Tyagi. In particular, peptides are relatively unstructured in solution, as opposed to nucleic acids, such as those taught in Tyagi, which self-hybridize in solution to form hair-pin structures between complementary internal sequences. As such, one of ordinary skill in the art would have had no reason to expect that attaching a dye near each end of a peptide might generate a reagent that would be useful for monitoring non-cleavage, covalent modifications to a peptide by non-FRET quenching.

Thus, without improperly relying upon the hindsight provided by the disclosure and claims of the above-referenced application, it is not seen how the teachings of any of these references could have provided one of ordinary skill in the art to use the dye pair of Tyagi to detect covalent modifications to a core molecular substrate.

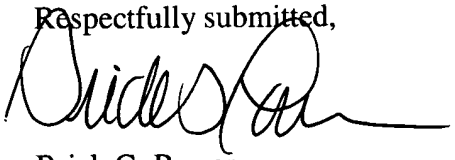
As no combination of teachings from Macala, Shultz, or Ventura, with those of Blumenthal and Odom or Tyagi supports a *prima facie* case of obviousness under 35 U.S.C. § 103(a), it is respectfully submitted that, under 35 U.S.C. § 103(a), the subject matter recited in each of claims 1-27 is allowable over the teachings of these references.

It is respectfully requested that the 35 U.S.C. § 103(a) rejections of claims 1-27 be withdrawn and that each of these claims be allowed.

CONCLUSION

It is respectfully submitted that each of claims 1-27 is allowable. An early notice of the allowability of each of these claims is respectfully solicited, as is an indication that the above-referenced application has been passed for issuance. If any issues preventing allowance of the above-referenced application remain which might be resolved by way of a telephone conference, the Office is kindly invited to contact the undersigned attorney.

Respectfully submitted,



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